IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Applicant(s):	Tereba et al.	**Docket No.:**	016026-9043-US01
Serial No.:	10/694,475	**Group Art Unit:**	1639
Filing Date:	October 27, 2003	**Examiner:**	Christopher M. Gross
Title:	SIMULTANEOUS ISOLATION AND QUANTITATION OF DNA		

DECLARATION OF REX BITNER UNDER 37 CFR § 1.132

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

I, Rex Bitner, do hereby declare and state the following:

1. I have served as the Technology Manager of the Genetic Analysis R&D group at Promega Corporation, Madison, WI, since 2003. From 1997-2003, I worked as a Senior Scientist and Senior Project Manager of R&D at Promega Corporation. I hold a B.S. in Biology from The University of Washington, Seattle, WA and a Ph.D. in Genetics from The University of California, Davis, CA. A copy of my curriculum vitae is attached as Exhibit A.

2. I am a joint inventor of at least some of the claimed subject matter of the above-identified patent application. I make this declaration in support of prosecution of the present application before the U.S. Patent and Trademark Office.

3. I have read and understand the invention as disclosed in the present application, including the invention described by the presently pending claims. I have also reviewed the July 31, 2007 Office Action. I understand that each of claims 44-52, 54, 55, and 58-68 is rejected as being unpatentable (i.e., obvious) over Kleiber et al. (WO 96/41811), Huber et al. (1993 Nuc. Acids Res. 21:1061-1066), and Vogelstein et al. (1979 PNAS 76:615-619).

4. Claims 44-52, 54, 55, and 58-68 are directed to methods of isolating a defined and consistent amount of DNA from multiple samples by choosing the amount of DNA to be

isolated, choosing an amount of a silica containing solid support needed to isolate the defined amount of DNA, such that the amount of DNA in the samples is greater than the binding capacity of the solid support, and contacting each sample with the solid support under conditions that allow isolation of the defined and consistent amount of DNA.

5. DNA IQ™ System is the term that Promega Corporation, the assignee of the instant application, uses with its customers when referring to methods for isolating a defined and consistent amount of DNA from multiple samples, as described throughout the application and as summarized in paragraph 4, above.

6. The concept underlying the methods of the invention, which focus on isolating just a portion of DNA that may be present in a sample, represents a complete departure from prior art methods such as those described in Kleiber et al. and Vogelstein et al., which focus on maximizing DNA yield. For example, Kleiber et al. discusses the relatively high yields obtained by their methods. (Please see, for example, page 12 of Kleiber et al.). Similarly, Vogelstein et al. emphasizes that binding DNA to glass from dissolved agarose "is rapid, convenient, and nearly quantitative." (Vogelstein et al., p. 618, first column, last line to second column, first line, emphasis added). Huber et al., rather than isolating a defined and consistent amount of DNA, describes using high performance liquid chromatography (HPLC) to fractionate relatively small (i.e., <500 bp) DNA fragments such as restriction fragments and PCR products according to size.

7. The ability to isolate a defined and consistent amount of DNA from multiple samples using the methods of the invention simplifies processing, reduces the amount of time needed to process samples, and increases sample throughput. Because DNA samples prepared using the method of the invention contain a select defined and consistent amount of DNA, the isolated DNA can be used directly in downstream applications requiring an amount of DNA within a particular range.

8. The importance of increased sample throughput in isolating DNA cannot be overstated. For example, because prior art methods of isolating DNA from samples were very labor-intensive and cost about $1000 per test, a backlog of several hundred thousand samples from rape victims awaited processing in 1999 when the patent application for the present invention

was submitted. Thus, samples containing evidence that may have been useful in identifying sex offenders go unprocessed.

9. Attached as Exhibit B is an article entitled "Robotic Extraction of Mock Sexual Assault Samples using Biomek® 2000 and the DNA-IQ™ System", authored by Susan Greenspoon and Jeff Ban of the Virginia Division of Forensic Science, which appeared in the February 2000 issue of Profiles in DNA.

10. Greenspoon and Ban describe how the DNA IQ™ System, used in conjunction with the Biomek® 2000 robotics system, was able to isolate uniform amounts of DNA from mock sexual assault samples containing dilutions of semen of from 1:10 to 1:200 on a ½, ¼, or 1/8 portion of a swab (See p. 4, Mock Sexual Assault Samples). The DNA thus isolated from samples having widely varying DNA content was used directly in PowerPlex® 1.1 System, a DNA amplification reaction used in genetic identity testing, and produced uniform results.

11. Other experts in the field have recognized the importance of the instantly claimed methods isolating DNA for use in molecular biological methods such as amplification for genetic identity testing.

12. Dr. Weimin Sun, Scientific Director in the Molecular Genetics Department of Quest Diagnostics Nichols Institute, San Juan Capistrano, CA, evaluated the DNA IQ™ System for use in clinical samples and found that the methods yielded consistent quantities of DNA despite significant variations in the starting material, which afforded satisfactory performance in downstream applications. (See correspondence form Dr. Sun to Mr. David Phelps, Exhibit C).

13. Kim Gorman, then President of Paternity Testing Corporation, Columbia, MO, reported that the DNA IQ™ System was used to extract DNA from buccal swabs in a 96 well format. (See correspondence from Ms. Gorman to Mr. Phelps, Exhibit D). Ms. Gorman noted that buccal swabs vary greatly in DNA content, and also noted that DNA multiplexes (amplification of multiple DNA loci in a single reaction) are concentration sensitive. Despite these challenges, Ms. Gorman further noted that there was no need to quantify the DNA prepared using the DNA IQ extraction method prior to use in a multiplex reaction. Ms. Gorman reported that, using the DNA IQ™ System, the total time required to process 96 samples was reduced from 6 or 7 hours

using their traditional extraction method to about 3 hours, and hands on time by analysts was reduced from more than 4 hours to less than 30 minutes.

14. Jeffrey Ban, Section Chief of Forensic Biology at the Virginia Department of Criminal Justice Services, Division of Forensic Science, reported that, using the DNA IQ™ System in conjunction with Beckman Coulter Biomek® 2000 Workstation or other similar instrumentation, could greatly increase a laboratory's throughput capabilities, thus permitting the Forensic DNA community to provide better service to law enforcement agencies (See correspondence from Mr. Ban to Mr. Phelps, Exhibit E).

15. In addition, Promega has been contacted by numerous law enforcement agencies that ultimately used the DNA IQ™ System to analyze forensic samples from crime scenes. For example, the Royal Canadian Mounted Police requested assistance in processing samples collected from a hog farm in British Columbia, where the partial remains of at least 26 murder victims were found. The DNA IQ™ System was also used to isolate DNA for genetic identity testing of victims at Ground Zero and human remains found in mass graves in Bosnia.

16. In 2002, Promega received an R&D 100 Award for its DNA IQ™ System. Through the R&D 100 Awards program, sponsored by R&D Magazine, organizations receiving the awards are recognized for the most technologically significant products introduced into the marketplace.

17. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements and the like so made are punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: October 30, 2007

Rex Bitner

Rex Bitner

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QUALIFICATIONS SUMMARY

Over twenty years of industrial experience in biotechnology research and product development at Promega (Madison, WI), Amersham/Pharmacia Biotech (now GE Healthcare), (Milwaukee, WI), and 3M (St. Paul, MN). Four years of postdoctoral research in molecular biology at the University of Colorado, Boulder and the University of California, Davis. Broad, in-depth knowledge of molecular biology, solid phase purification of biological materials, and all aspects of product development in an ISO9001: 2000 environment. Experienced in technology management, project leadership and management, supervision of a BL3 laboratory, and the development, launch and care for biotechnology products. Extensive experience in the creation, development and evaluation of intellectual property, including USPTO and foreign patent office actions, in person interviews with USPTO patent examiners, as well as participation in foreign patent opposition proceedings, both as proprietor and as an opposition party, and as an opposition member in the appeal of an invalidated patent.

PROFESSIONAL HISTORY

1997 - present **PROMEGA CORPORATION, Madison, Wisconsin**

Technology Manager, Genetic Analysis, R&D (2003 - present)

Technology Manager, Genetic Analysis: management of a laboratory group, development of separation technologies, intellectual property, and purification products for the biotechnology marketplace and clinical laboratory market, with particular emphasis on automation of nucleic acid purification products for use in genomics, high throughput pharmaceutical drug screening, and clinical diagnostics. Intellectual property management has included USPTO and foreign patent office actions, in person interviews with USPTO patent examiners, phone interviews with USPTO and foreign patent examiners, and participation in European patent opposition proceedings, both as proprietor and as an opposition party, including as an opposition member in the appeal of an invalidated patent. The efficient integration of foreign patent filing strategies and licensing of intellectual property within Promega's Genetic Analysis business strategy has been a central responsibility.

Senior Scientist, Senior Project Manager, R&D (1997 - 2003)

Development of pH dependent ion exchange purification systems using both column and paramagnetic particle purification methods in robotic workstations, with additional emphasis on automated cell concentration and magnetic clearing of cellular lysates in 96-well walkaway automated DNA purification (particularly using Beckman BioMek® FX and Tecan Genesis® robotic platforms). Products developed for genomic DNA purification from human whole blood and tissues (including R&D Magazine's R&D 100 award winning DNA-IQ™(for 2002), plant materials, and DNA purification from food ingredients for use in the quantitative detection of genetically modified organisms (GMO) in food. Additional experience with DNA sequencing automation, RNA purification, and PCR cleanup, particularly using Beckman, Tecan and Thermo-Electron Labsystems robotic platforms.

Project leader for Promega products, in an ISO9001: 2000 environment, including:

Wizard® Genomic, 10ml blood	A1620	Wizard® Magnetic DNA Purification for Food	FF3751
Wizard® Magnetic 96 DNA Plant	FF3761	MagneSil® Blood Genomic, Max Yield	MD1360
MagneSil® ONE, Fixed Yield	MD1370	MagneSil® KF, Genomic System	MD1460
PureYield™ RNA Midi-Prep System	Z3741	PureYield™ Plasmid Midi-Prep System	A2495

PROFESSIONAL HISTORY, continued

1995 - 1997 **AMERSHAM PHARMACIA BIOTECH INC., Milwaukee, Wisconsin.**

Senior Research Scientist (1995 - 1997)

Senior Research Scientist and Project Leader responsible for nucleic acid purification products for the biotechnology laboratory: development of novel separations matrices and processes, formulation and execution of project plans, maintenance of timelines and scheduling, and supervision of personnel on several project teams. Research and development of new products for the molecular biology marketplace, particularly in the areas of proprietary purification products, anion exchange chromatography, and solid phase extraction and immobilization of nucleic acids. Responsibilities included the management of personnel, timelines and ISO 9001 documentation of product development.

1982 - 1994 **3M COMPANY, St. Paul, Minnesota.**

Research Specialist (1984 - 1994)

Identifying, planning and pursuing molecular biology programs of interest to 3M businesses. Research programs involved diverse product objectives: Development of 3M's Rapid Attest™ biological/sterilization monitor product, GMP purification of bovine phosphophoryn proteins (for bone repair), genetic manipulation of bacteria to produce specialty chemicals (*meta*-hydroxyphenylacetylene, and aromatic compounds useful in laser dyes), cDNA cloning of mammalian genes for drug discovery screening, surface immobilization of nucleic acids onto ceramic oxide/3M Empore™ (PTFE) membranes (for use in DNA blotting, hybridization and sequencing), solid phase extraction of DNA for automated sequencing, solid phase extraction of DNA from human blood plasma for use in PCR, protein immobilization on azlactone functionalized porous beads (including 3M Emphaze™ beads and network beads), and cloning of stress protein genes from bacteria associated with periodontal disease. Development and implementation of DNA purification technologies, using a variety of ceramic matrices for solid phase extractions. Over ten years experience supervising a biosafety level 3 containment laboratory.

Other responsibilities included evaluation of both internal and external research proposals and intellectual property. Additional responsibilities as Institutional Biosafety Officer, OSHA blood-borne pathogen safety officer, and as scientific advisor in the development of and compliance with Minnesota State (Environmental Quality Board) regulations governing recombinant organisms.

Senior Biologist (1982 -1984)

Responsibilities included setting up and staffing a recombinant DNA laboratory, evaluation of outside business proposals and intellectual property issues, and initiation of new research programs in molecular biology: gene expression in *Bacillus subtilis*, and R&D of 3M's Rapid Attest™. Additional responsibilities: initiation of university research contracts and management of laboratory personnel.

1978 - 1982 **UNIVERSITY OF COLORADO, Boulder, Colorado.**

Postdoctoral research: Dr. Peter L. Kuempel, Dept. of Molecular, Cellular, and Developmental Biology: Termination of chromosome replication in *E. coli*.

PROFESSIONAL HISTORY, continued

1974 - 1978 **UNIVERSITY OF CALIFORNIA, Davis, California.**

Postdoctoral research: Dr. Gordon G. Edlin, Dept. of Genetics (1978).
Instructor: Department of Genetics (1977).

1974 **UNIVERSITY OF WASHINGTON, Seattle, Washington**
Post-graduate Research Assistant: Dr. Jonathan A. Gallant, Dept. of Genetics

EDUCATION

Ph.D. in Genetics, 1978
University of California, Davis, California

B.S. in Biology, *cum laude*, 1974
University of Washington, Seattle, Washington

PATENTS

Rex M. Bitner, Mark A. Denhart, Don Smith. US Utility Application: WO2007/103485. Small RNA Purification.

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Rex M. Bitner, Michelle Mandrekar, Don Smith, Douglas H. White. WO2007005613, US2007015191. Network of Buoyant Particles for Biomolecule Purification and Use of Buoyant Particles or Network of Buoyant Particles for Biomolecule Purification.

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Allan M. Tereba, Rex M. Bitner, Susan C. Koller, Craig E. Smith, Daniel D. Kephart, Steven J. Ekenberg. US 6,673,631 B1, CA 2379503, EP 1204741, EP 1510578, DE 60015148 and WO0114590. Simultaneous Isolation and Quantitation of DNA.

Rex M. Bitner, Jacqui Sankbeil, Braeden L. Butler, Douglas H. White, Craig E. Smith. US 7,078,224, US 6,284,470, EP1179058, EP1341910, EP1621618, and WO0070040. Cell Concentration and Lysate Clearance Using Paramagnetic Particles.

Craig E. Smith, Diana L. Holmes, Daniel J. Simpson, Jehoshua Katzenhendler, Rex M. Bitner, Josephine C. Grosch. US 6,806,362, US 6,310,199, EP1179057, AU5126100 and WO0069872. pH Dependent Ion Exchange Matrix and Its Use in the Isolation of Nucleic Acids.

Craig E. Smith, Diana L. Holmes, Daniel J. Simpson, Jehoshua Katzenhendler, Rex M. Bitner, Josephine C. Grosch. US 6,376,194, US 6,270,970, EP1179056, AU4841500 and WO0070041. Mixed Bed Solid Phase and Its Use in the Isolation of Nucleic Acids.

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Craig E. Smith, Donald A. Creswell, Rex M. Bitner, Douglas H. White, Braeden L. Butler, Scott A. Lesley. US 6,194,562, EP1071695, CA 2329067, and WO 9954340. Endotoxin Reduction in Nucleic Acid Purification.

Rex M. Bitner, Chan-Wha Kim, and Michael G. Williams. EP647232B1, WO9400464. 3M, St. Paul, MN. Deproteinization with azlactone-functional supports.

Rex M. Bitner and Eric F. Funkenbusch. EP0391608. Applicant: Minnesota Mining and Manufacturing Company, St. Paul, MN. Metal oxide supports for nucleic acids.

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Koller, S., H. Shenoi, R. Bitner, 2005. "Purifying Genomic DNA from Plant Tissue on Automated High-Throughput and Moderate-Throughput Platforms" JALA 10: 155-162.

Bitner, R. M. and S.C. Koller. 2004. "Automated high throughput purification of genomic DNA from plant leaf or seed using Promega's MagneSil® paramagnetic particles" Proceedings of SPIE, Microarrays and Combinatorial Techniques: Design, Fabrication and Analysis II, Vol 5328, p. 78-86.

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Smith, C., P. Otto, R. Bitner and G. Shiels. 2003. "Chapter 9: DNA Purification", in PCR Primer: A Laboratory Manual 2nd Edition, editors: Carl W. Dieffenbach and Gabriela S. Dveksler, Cold Spring Harbor Laboratory Press, ISBN 0-87969-6532, p.87-115.

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Bitner, R. and S. Koller. 2001. "Automation of DNA Extraction from Food and Plants Using MagneSil™ Paramagnetic Particles" Genomics and Proteomics Technologies, *Proceedings of SPIE* Vol 4264 p. 9-16.

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Lin, L., R. Bitner and G. Edlin. 1977. "Increased Reproductive Fitness of *Escherichia coli* Lambda Lysogens" J. Virol. *21*: 554-559.

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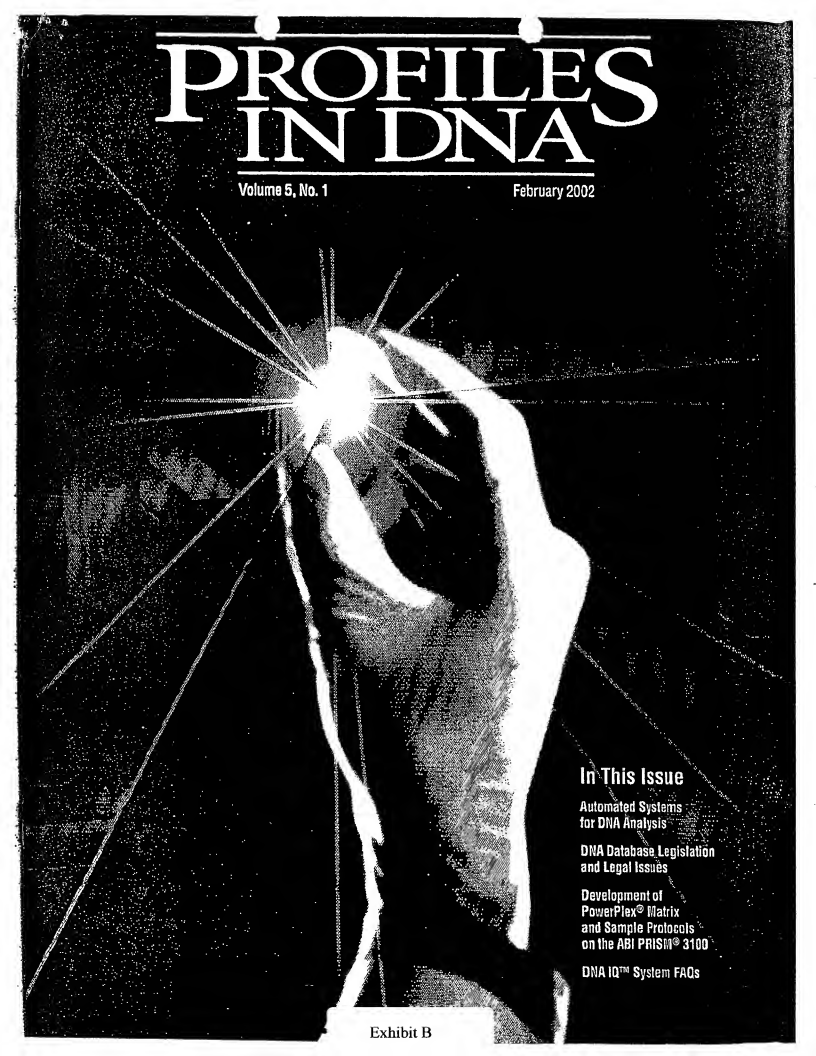
PROFILES IN DNA

Volume 5, No. 1 February 2002



In This Issue

Exhibit B

Robotic Extraction of Mock Sexual Assault Samples Using the Biomek® 2000 and the DNA IQ™ System

By Susan Greenspoon and Jeff Ban
Virginia Division of Forensic Science, Richmond, Virginia

The Biomek® 2000 used in conjunction with the DNA IQ™ System has proven to be an efficient robotic system designed to handle the challenges of routine casework samples.

INTRODUCTION

Forensic scientists are routinely faced with the challenge of isolating DNA from a large array of tissue and cell types. The variety of substrates upon which cellular material has been deposited, some of which may contain inhibitors of PCR, can make the process more difficult (1). Therefore, any robotic system applied to the extraction of forensic casework samples must be robust enough to address these variations. The Biomek® 2000 used in conjunction with the DNA IQ™ System(2) has proven to be an efficient robotic system designed to handle the challenges of routine casework samples.

The DNA IQ™ System uses silica-coated magnetic beads to separate DNA from cellular debris. Cells are lysed in a powerful lysis buffer, and the lysate is mixed with the magnetic beads. The beads saturate at approximately 100ng of bound DNA, and the excess DNA is removed by pipetting. Once bound to the magnetic resin, the DNA is pipetted and vigorously shaken several times in wash buffer, then eluted using heat. The Biomek® 2000 is equipped with a magnetic plate, a shaking platform and a thermal exchange unit to perform these necessary steps.

CONTAMINATION STUDIES

A number of exploratory and validation studies have been performed on the Biomek® 2000/ DNA IQ™ System to evaluate the viability of this automated system for use with forensic samples. Fundamental questions needed to be answered before moving ahead with extensive validation work. First, does the robotic, open-plate format cause contamination? To answer this question, two sample formats were used repeatedly, and extracted samples were analyzed to test

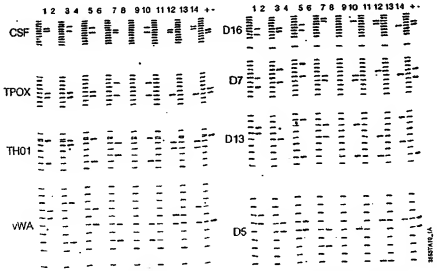


Figure 1. Amplified DNA samples using the PowerPlex® 1.1 System from the 88-sample checkerboard contamination study. Numbers 1–14 indicate sample numbers, "+" indicates positive control (K562), and "–" indicates negative control. Amplified DNA was analyzed by electrophoresis using a 6% polyacrylamide gel (Gibco BRL) for 2 hours at 50 watts. Both the 585nm scan (left panel) and the 505nm scan (right panel) are shown (gel imaging performed using a Hitachi FMBIO® instrument).

for contamination. The first is the zebra-stripe format test: alternating columns of samples containing an abundant source of DNA with columns containing reagent blanks (8 sample wells per column). Therefore, a column of samples containing abundant DNA was processed adjacent to a column of reagent blanks in a striped pattern on the plate. The samples containing DNA were bloodstains cut into 5mm² squares. DNA was eluted from the magnetic beads into 100µl of sterile water, then quantified, amplified, and typed using the PowerPlex® 1.1 System(b/o) (2). The first two trials of this test detected some contamination. The software method used was modified to accommodate sample loading into a 96 deep-well plate in place of the more shallow Greiner plate and to remove an initial shaking step. A subsequent zebra stripe experiment showed no contamination with the 40 samples that were isolated.

The second contamination test is a checkerboard sample format: samples containing abundant DNA were alternated with reagent blanks in a checkerboard pattern across a 96 deep-well plate (Figure 1). All 128 samples (88 sample and 40 sample methods) tested negative for any detectable contamination.

MOCK SEXUAL ASSAULT SAMPLES

Sexual assault cases frequently constitute the majority of DNA cases received by a forensic laboratory. Presently, no robotic system is available that can separate sperm from non-sperm cells and thus perform a differential extraction (3) from start to finish. However, the first step of separating fractions can be performed manually. Subsequently, the DNA from E-cell (epithelial or non-sperm cell) lysates and sperm pellets can be extracted robotically, saving analysts a substantial amount of time. Any robotic extraction of sexual assault samples must at least be able to generate sample DNA, equivalent in both quality and yield to that generated by manual DNA extraction methods. Therefore, a thorough examination of the Biomek® 2000/DNA IQ™ System's ability to isolate DNA from sexual assault samples needed to be performed. The first step was to ascertain whether sperm cells could be successfully lysed and the DNA purified by the robotic system. Mock sexual assault samples were prepared using previously donated vaginal swabs and semen from a known donor, which was deposited onto sterile cotton swabs in 1:2 and 1:4 dilutions. The E-cells were lysed manually, the sperm cells pelleted, and a portion of the lysates and the entire sperm pellets were loaded onto the Biomek® 2000 for DNA extraction. DNA was eluted off the magnetic beads into 100µl of sterile water. High-quality DNA was obtained and typed accurately using the PowerPlex® 1.1 System (data not shown).

Once it was demonstrated that the Biomek® 2000/DNA IQ™ System could successfully complete the differential extraction process with the E-cell lysates and intact sperm, the next question addressed was whether this automated system could produce DNA of comparable quality and yield to that produced by manual extraction. A comparative study was designed to measure the performance of the Biomek® 2000/DNA IQ™ System with respect to manual extraction of similar if not identical samples. Samples were prepared in the following manner:

1. Sets of vaginal swabs from 5 different donors were selected.
2. Duplicate mock sexual assault swabs were prepared using semen from a single donor at the following dilutions: 1:10, 1:100, 1:1,000 and 1:10,000 for three sets, 1:10, 1:100, 1:200 and 1:400 for one set and 1:100, 1:200, 1:400 and 1:800 for the last set.
3. Once dried, the swabs were cut into 1/2, 1/4 and 1/8 portions.
4. The E-cells were lysed and the sperm cells pelleted and washed.
5. The samples were split evenly with one half going to an analyst to complete the extraction manually and the other loaded onto the Biomek® 2000 for a robotic DNA extraction.

Yields and quality of the DNA from the sperm fractions processed by the Biomek® 2000/DNA IQ™ System were comparable and frequently superior to those obtained by manual extraction (Figure 2). In less experienced hands, the Biomek® 2000/DNA IQ™ System clearly outperformed the manual extraction (data not shown). Results obtained by experienced users were equivalent to those achieved with the robot. Therefore, the Biomek® 2000/DNA IQ™ System is not only capable of outperforming its human counterpart, but it also delivers a more consistent product.

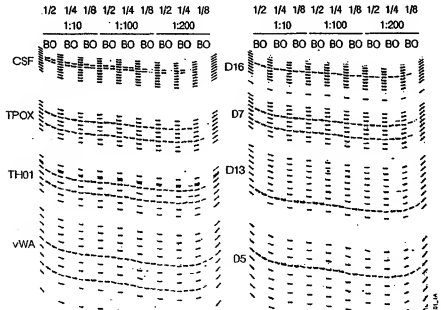


Figure 2. PowerPlex® 1.1 mock sexual assault comparison study. Duplicate samples were extracted manually (indicated by an "O" above the lane, for organic) or robotically (indicated by a "B" above the lane, for Biomek® 2000). Semen dilutions (1:10, 1:100 and 1:200) for each set of swabs are indicated above the corresponding six sample lanes, which contain DNA extracted from the indicated swab portion (1/2, 1/4 or 1/8). Amplified DNA was separated by electrophoresis in a 6% polyacrylamide gel (Gibco BRL) for 2 hours at 50 watts. Both the 585nm scan (left panel) and the 505nm scan (right panel) are shown (gel imaging performed using a Hitachi FMBIO® instrument).

The maximum sample volume for use with the 96 deep-well plate is limited to 100µl. Sperm cells are typically in a pellet of 50µl and therefore unaffected by the volume limit. Since the E-cell lysate is usually in a volume of 500µl, it was important that the yields from 100µl of E-cell lysate from the 1/2, 1/4 and 1/8 swab portions be sufficient for all DNA typing needs. Total yields of E-cell DNA extracted on the robot were calculated (Figure 3), and sufficient E-cell DNA could be obtained using robotic extraction methods. In fact, 1/8 swab portions provided more than enough DNA for an E-cell DNA profile.

The amount of time saved by using the Biomek® 2000/DNA IQ™ System to extract the forensic samples can be substantial. The time it takes to complete the organic extraction manually for a single sample is 5 hours and 5 minutes (after E-cells have been lysed, and sperm cells pelleted and washed). Of course, additional samples will lengthen the amount of time proportionately. In comparison, the robot takes 1 hour and 15 minutes to extract the DNA from 40 samples and 1 hour and 50 minutes to extract 88 samples. Therefore, the absolute minimum amount of time saved is 3 hours and 50 minutes or half of a day.

EXTRACTION OF OTHER CELL AND TISSUE TYPES

Since a variety of cells and tissue types are encountered in routine forensic casework, the Biomek® 2000/DNA IQ™ System was evaluated to determine its capability to isolate DNA from a variety of sources. Dried bloodstains, E-cell lysates, intact sperm cells, muscle, heart, brain, liver and buccal swabs were extracted using the Biomek® 2000/DNA IQ™ System and successfully typed using the PowerPlex® 1.1 System (data not shown).

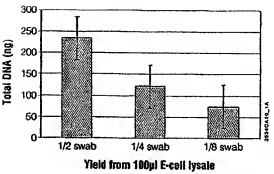


Figure 3. Bar graph depicting the total yield of E-cell DNA generated from extraction on the Biomek® 2000 robot using 100µl of lysate. Yields were determined by measuring DNA concentration using the QuantiBlot® kit.

CONCLUSION

A completely automated system for extraction of sexual assault samples is currently not available. However, once the E-cells have been separated from the sperm cells, robotic DNA extraction using the Biomek® 2000/DNA IQ™ System can be accomplished. Moreover, when sperm DNA is limited, the Biomek® 2000/DNA IQ™ System generates DNA of similar, and sometimes better, quality and yield than that obtained by manual extraction of a duplicate sample. Because of the adaptability of the Biomek® 2000/ DNA IQ™ System, this instrument has the potential to handle future applications of emerging cell separation technologies. It may be possible on a robotic platform, to separate sperm cells from non-sperm cells with the use of an anti-sperm antibody conjugated to a magnetic bead. One can envision a completely automated system where both cell separation and DNA extraction are performed on the same robot. The Biomek® 2000/DNA IQ™ System may be uniquely poised to proceed with that application when the technology becomes available.

The time saved when compared with manual extraction, as well as the ability to extract a variety of tissue and cell types, makes the Biomek® 2000/DNA IQ™ System attractive for casework applications. Further validation work on the Biomek® 2000/DNA IQ™ System must be performed in order to complete our evaluation and validation prior to its application for forensic casework. Although no contamination of the samples was detected after modifying the method and changing the format, special circumstances might require the use of manual extraction methods, for example, when an evidentiary sample may be completely consumed due to limited available material.

ACKNOWLEDGMENTS

We would like to acknowledge the participation of other members of the Virginia Division of Forensic Science: Beth Ballard, Missy Baisden, Brian Covington, Shelley Smith and Colleen Young. We would also like to thank Allan Tereba and Dan Kephart at Promega Corporation for all their hard work at making the technology and this study possible.

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March 4, 2002

David Phelps
Promega
Genetic Identity
2800 Woods Hollow Drive
Madison, WI 53711

Dear Mr. Phelps,

We have evaluated DNA IQ system for isolation of DNA from clinical specimens (whole blood and buccal swabs). The IQ system showed satisfactory performance on both types of samples. It is highly tolerant to the variability of sample input quantity. It also demonstrated high consistency in the quantity of product DNA. This feature is extremely desirable to us as buccal swab samples can have significant variations in starting material affected by collection procedure and skills. In addition, DNA samples isolated using the DNA IQ system have shown satisfactory performance in the downstream applications and have excellent stability as well.

If there is any question in regards to our experience with the DNA IQ system, please do not hesitate to contact me.

Sincerely,

Weimin Sun, Ph.D., ABMG.
Scientific Director
Molecular Genetics Department
Quest Diagnostics Nichols Institute
33608 Ortega Highway
San Juan Capistrano, CA 92690
(949) 728-4498 (voice)
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Paternity Testing Corporation
Fast, Confidential DNA *Analysis*

February 25, 2002

David Phelps
Promega, Genetic Identity
2800 Woods Hollow Drive
Madison, WI 53711

VIA FACSIMILE: (608) 273-6455

Dear Mr. Phelps:

Lisa Lane requested that I send you a brief letter about our experience using the DNA IQ extraction method.

PTC has been extremely interested in automating DNA extractions from buccal swabs. Because of the nature of buccal swabs it has historically been extremely difficult if not impossible to work with them in a 96 well automated format. Swabs absorbed liquid and had to be spun through a basket in order to recover the lysis buffer. Swabs had to be removed by hand one at a time. There were many transfers in and out of centrifuges. It was a very labor intensive process to carry out the extractions. It was possible to automate the extractions, but that required large volumes and could not be carried out in a 96 well format.

Our goal was to be able to put a buccal swab into a 96 well tray and never have to touch it again. DNA IQ allows for extractions to be carried out in the 96 well format from the time the specimens are placed into a 96 well deep well tray until the DNA is transferred to the amplification tray.

DNA IQ also has the advantage of eliminating the need to quantify the DNA. Buccal swabs vary greatly in DNA content. Since the DNA multiplexes are somewhat concentration sensitive, it was necessary to get a rough quantification of the amount of DNA present then diluting to appropriate volumes. There is no need to quantify the DNA using the DNA IQ extraction method. The concentration of DNA is consistent from well to well. By using 1µl of the isolated DNA we are able to get consistent results from virtually all specimens.

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DNA IQ is also much faster than most extraction methods. It takes approximately 3 hours from the time the swabs are placed into the tray until the DNA is pipetted into the amplification tray. There is less than 30 minutes of analysts time involved in the entire process. Using our traditional extraction method it takes 6 or 7 hours to prepare 96 samples for amplification and more than 4 hours of actual hands on time by the analysts.

Our lab has nearly completed the final stages of testing and validation of DNA IQ. We plan to be online using DNA IQ in March.

Yours truly,

Kim Gorman

Kim Gorman
President

COMMONWEALTH of VIRGINIA

DEPARTMENT OF CRIMINAL JUSTICE SERVICES

DIVISION OF FORENSIC SCIENCE
CENTRAL LABORATORY
A Nationally Accredited Laboratory

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February 25, 2002

David Phelps
Promega Corporation
2800 Woods Hollow Road
Madison, WI 53711-5399

Dear Mr. Phelps,

In order to aid the DNA examiner in the extraction of casework samples, the Virginia Division of Forensic Science, Forensic Biology Section, for the past 6 months has worked with scientists and engineers from the Promega Corporation and Beckman Coulter to evaluate, modify and validate the Beckman Coulter Biomek® 2000 Workstation in conjunction with the Promega DNA IQ™ Isolation System. Currently each DNA examiner spends between 6 and 8 hours purifying the DNA from an average sexual assault case consisting of 3 to 5 evidence samples. Because of the adaptability of the Promega DNA IQ™ Isolation System the DNA purification can be automated permitting the user to walk away once the samples are setup in the robot.

In the time it would takes 4 DNA case examiners (i.e. between 24 and 32 person hours) to manually purify the DNA from evidence samples from 3 to 5 cases, using an organic extraction procedure, the robot can accomplish in under 2 hours due to the speed of the Promega DNA IQ™ purification process paired with the Beckman Coulter Biomek® 2000 Workstation. Therefore, with as little as 5 to10 minutes of a technician's time to setup the instrument, the Virginia Division of Forensic Science, Forensic Biology Section can improve the turnaround time of cases with minimum impact on the casework examiner.

Utilizing the chemistry of the Promega DNA IQ™ Isolation System in combination with the Beckman Coulter Biomek® 2000 Workstation or similar instrumentation can greatly increase a laboratory's throughput capabilities for analyzing forensic casework samples. Thus in turn

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permitting the Forensic DNA community to provide a better service to the law enforcement agencies throughout the United States to help solve crimes against persons and property.

Yours sincerely,

Jeffrey D. Ban
Forensic Biology
Section Chief